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Durability of the anti-erosive effect of surfaces sealants under erosive abrasive conditions

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Abstract: **OBJECTIVE:** To test the durability of sealants applied for prevention of erosive dentine mineral loss under erosive/abrasive conditions. **METHODS:** Forty-eight bovine dentine samples doped with (32)P were randomly allocated to four groups (1-4). All samples performed a de- and remineralizations pre-cycling (6 × 1 min erosion in HCl: pH 3.0, mean time and overnight immersion in artificial saliva) for 1 day. Sealing was done as follows; (1) unsealed, (2) Seal Protect, (3) K-0184 (experimental sealer) and (4) OptiBond FL. After sealing, samples were immersed in HCl for 3 h (baseline measurement). Then, the following erosive/abrasive and remineralisations cycling was performed for 8 days: 3 h/day erosion with HCl, 600 brushing strokes/day and storage in artificial saliva for the rest of the day. Sealer permeability was evaluated by assignation of (32)P in the acid used for the erosive attacks. **RESULTS:** At baseline, the significantly highest dentine loss was observed for the unsealed control group, while the mineral loss was not statistically significantly different between the sealed groups 2 and 3. At all days of the erosive/abrasive and remineralizations cycling and cumulatively the significantly highest mineral loss was observed for group 1, while the significantly lowest mineral loss was observed for the samples sealed with Seal Protect (group 2) and K-0184 (group 3). In all groups, no significant increase in the (32)P release was observed. **CONCLUSION:** Surface sealants are able to reduce the erosive dentine mineral loss and maintain this erosion-preventing efficacy over the whole duration (simulating 8 month in-vivo) of the erosive/abrasive cycling.

DOI: <https://doi.org/10.3109/00016357.2012.757361>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-87911>

Journal Article

Accepted Version

Originally published at:

Wegehaupt, Florian J; Tauböck, Tobias T; Attin, Thomas (2013). Durability of the anti-erosive effect of surfaces sealants under erosive abrasive conditions. *Acta Odontologica Scandinavica*, 71(5):1188-1194.

DOI: <https://doi.org/10.3109/00016357.2012.757361>

Durability of the anti-erosive effect of surfaces sealants under erosive abrasive conditions.

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Short title: Durability of surface sealants

Word count abstract: 249

Tables: 1

Abstract:

Objective: To test the durability of sealants applied for prevention of erosive dentine mineral loss under erosive/abrasive conditions.

Methods: 48 bovine dentine samples doped with ^{32}P were randomly allocated to four groups (1-4). All samples performed a de- and remineralisations precycling (6 x 1 min erosion in HCl: pH 3.0, mean time and over night immersion in artificial saliva) for one day. Sealing was done as follows; 1: unsealed, 2: Seal&Protect, 3: K-0184 (experimental sealer) and 4: OptiBond FL. After sealing, samples were immersed in HCl for 3 h (baseline measurement). Then, the following erosive/abrasive and remineralisations cycling was performed for 8 days: 3 h/day erosion with HCl, 600 brushing strokes/day and storage in artificial saliva for the rest of the day. Sealer permeability was evaluated by assignation of ^{32}P in the acid used for the erosive attacks.

Results: At baseline, the significantly highest dentine loss was observed for the unsealed control group, while the mineral loss was not statistically significantly different between the sealed groups 2-3.

At all days of the erosive/abrasive and remineralisations cycling, and cumulative the significantly highest mineral loss was observed for group 1, while the significantly lowest mineral loss was observed for the samples sealed with Seal&Protect (group 2) and K-0184 (group 3). In all groups, no significant increase in the ^{32}P release was observed.

Conclusion: Surface sealants are able to reduce the erosive dentine mineral loss and maintain this erosion-preventing efficacy over the whole duration (simulating 8 month *in-vivo*) of the erosive/abrasive cycling.

Key words: dentine, durability, erosion, surface sealant, erosive/abrasive cycling

Introduction:

Traditionally, the prevention of erosive tooth wear or erosion associated dental hard tissue softening [1,2] is mostly based on the use of topically applied fluoride formulations [3,4]. The use of topically applied fluoride compounds, such as sodium fluoride [5,6], amine fluoride [7], monofluorophosphate [5] or titanium tetrafluoride [7], results in a strengthening of the chemical resistance of dental hard tissues [2] or rehardening of erosively softened enamel or dentine [8]. In the recent years, studies testing new products or active ingredients, namely stannous chloride [9], cerium chloride [10] or CPP-ACP containing pastes [11], intended to prevent erosion associated dental hard tissue loss or softening, have been published. However, the effectiveness of reducing or preventing erosive tooth wear by fluoride or CPP-ACP is discussed controversially in the literature [12-14].

Furthermore, many of these preventive measures depend on patients' compliance. Thus, there is still uncertainty whether those measures are effective in prevention of erosive tooth wear or softening [15]. This is especially discussed for patients, which suffer from erosive tooth wear as a sign of a psychosocial pathological disorder, e.g. anorexia nervosa and bulimia nervosa [16,17]. Due to these findings, a more patient independent approach seems to be desirable. Furthermore, the durability of the anti-erosive effect of topically applied fluorides or the above mentioned new agents are limited, ranging from a few seconds (75 s) [18] up to few minutes (3.5 – 18 min) [12,19] after a single application.

Beside the prevention of erosive dental hard tissue loss or softening by topical application of various chemical compounds (fluoride, cerium- or stannous chloride or CPP-ACP), the erosion associated softening or loss of dental hard tissues could be prevented by hampering the contact of the erosion causing extrinsic or intrinsic acids with the dental hard tissues by means of a mechanical barrier on the enamel or dentine surfaces [20]. In 2000, Brunton et al. [21] suggested coating dentine with a resin-based dentine adhesive to prevent erosive/abrasive wear. Later, coating of teeth with a resin-based surface sealant showed a

significant protective effect against erosive/abrasive tooth wear under *in-vivo* and *in-vitro* conditions [22,23]. A recent *in-vitro* study found a significant reduction of erosive demineralisation by using surface sealants as a coating, even when samples were stored for 4 days in hydrochloric acid or 24 days in citric acid [24]. However, in that study only a demineralisation without an additional abrasive wear regime or remineralisation periods was performed.

Therefore, the aim of the present study was to evaluate, how long a protective effect of two different surface sealants (commercially available sealant Seal&Protect and an experimental sealant) and a dentine bonding agent (OptiBond FL, as gold-standard) will last under more *in-vivo* like conditions (de/remineralisation and tooth brushing abrasion). The hypothesis of the present study was that an increasing number of erosion/abrasion cycles would result in a reduced erosion-preventing efficacy of the three materials tested.

Materials and Methods:

Sample preparation

For the study, 48 dentine samples were prepared from freshly extracted bovine (age under 36 month) lower incisors. The teeth were sectioned at the cementum-enamel junction with a water-cooled diamante disc. The pulp tissue was removed from the roots with endodontic files.

Dentine drilling cores were gained with a trephine mill from the distal and mesial surface of each root. The inner diameter of the trephine mill amounts to 3 mm. The root surface was ground with abrasive paper (800, 1000, 1200, 2400 and 4000 grit; Water Proof Silicon Carbide Paper, Struers, Erkrath, Germany) with running tap water as coolant, to remove the cementum. To ensure that the dentine was fully denuded, all samples were checked under a light microscope.

The samples were irradiated at the “Atominstitut der Österreichischen Universitäten” (Vienna, Austria) with an exposure time of 85 min and a neutron flow of 0.17×10^{13} neutrons/cm² s to form radioactive ³²P.

After a 2-week storage periode, the drilled dentine cores were covered with a bonding resin and a flowable composite, leaving only the original surface dentine exposed. To achieve full coverage, samples were treated with Syntac Primer (Ivoclar Vivadent, Schaan, Lichtenstein) for 15 s (gently rub in), any excess was dispersed and thoroughly dried. Syntac Adhesive (Ivoclar Vivadent, Schaan, Lichtenstein) was applied, left for 10 s and again thoroughly dried using an air syringe. Next, the light curing bonding resin Heliobond (Ivoclar Vivadent GmbH, Ellwangen, Germany) was applied for 20 s, blown to a thin layer and light cured for 60 s. For the final dentine covering, the flowable composite (Orbi-Flow, ORBIS DENTAL, Münster, Germany) was applied and again light cured for 60 s. Finally, samples were checked with a stereomicroscope at a magnification of 40x for continuous covering of the cylinder sides and bottom and to ensure that the natural dentine surface was free of sealing materials. The samples were randomly allocated to four experimental groups (1 – 4; n = 12, each).

Surface sealing procedure

Before performing the surface sealing procedure, all samples of groups 1 - 4 were exposed to a de- and remineralisation precycling for one day. For this precycling, the samples were eroded six times per day for 1 min in hydrochloric acid (pH 3.0). The samples were immersed in artificial saliva for 1 hour between the erosive attacks and over night. The artificial saliva was prepared as described by Klimek et al. [25].

The dentine surfaces of the group 1 samples remained unsealed and served as positive controls. Group 2 samples were treated with Seal&Protect (DENTSPLY DeTrey GmbH, Konstanz, Germany), while the groups 3 samples were treated with K-0184 (experimental

sealer; DENTSPLY DeTrey GmbH, Konstanz, Germany). Following the manufactures instructions, the sealants were applied on the dentine surface and left undisturbed for 20 s. Afterwards, the remaining solvent was removed with a blow of an air syringe and the sealant was light cured for 10 s (Bluephase, Ivoclar Vivadent, Liechtenstein; Mode: HIP, 1200 mW/cm²). A second layer of sealant was applied, the solvent was again removed using an air syringe and again light cured for 10 s.

The samples in group 4 were sealed with OptiBond FL (Kerr, Orange, United States). The dentine was etched with phosphoric acid (37%) for 15 s, then rinsed for 15 s. After rinsing, the dentine was carefully dried using an air syringe for 3 s and the Primer was applied with light brushing motion for 15 s. Thereafter, the Primer was air dried for 5 s and the adhesive was applied with a light brushing motion for 15 s. Light curing was performed for 20 s (Bluephase, Ivoclar Vivadent; Mode: HIP, 1200 mW/cm²) after thinning the adhesive using an air syringe for 3 s.

Erosive, abrasive and remineralisation cycling

Directly after sealing the dentine surface, each sample was immersed in hydrochloric acid (pH 3.0; 10 ml per sample) for 3 h (Baseline measurement of sealer permeability).

Subsequently, the samples were exposed to the following erosive, abrasive and remineralisation cycle daily, for a total of 8 days: For simulation of toothbrush abrasion, the samples were brushed with 600 brushing strokes (BS) with toothpaste slurry in an automatic brushing machine applying reciprocating linear motion to the toothbrushes (ParoM43, Esro AG, Thalwil, Zürich, Switzerland). The brushing machine was adjusted to a constant brushing frequency of 120 strokes per minute and a constant brushing load of 2.5 N. The toothpaste slurry was prepared by mixing 300 ml artificial saliva and 100 ml toothpaste (elmex, Gaba, Münchenstein, Switzerland). To simulate the erosive attack, the samples were immersed in 10 ml hydrochloric acid (pH 3.0) each at 37 °C under constant motion for 3 h. These acids were

then each analysed to determine the amount of apatite in suspension and thereby evaluate possible changes in the sealer permeability. The rest of the day and over night, the samples were stored in artificial saliva for remineralisations purposes.

Radiochemical analysis

The dentine wear (μg) was quantified by assignation of ^{32}P in the acid of the baseline erosive attack and the erosive attacks of the days 1 – 8. Specifically, this was accomplished by determining the Cherenkow radiation and comparing this radiation with the radiation of known amounts of apatite. The respective laboratory procedure have been described in detail by Schmidlin et al. [26].

Statistical Methods:

Data were coded in EXCEL and analyzed with SPSS Version 16. Descriptive statistics such as mean, standard deviation (SD), median and interquartile range (IQR) and 95% confidence interval (95%CI) were computed for groups 1 – 4, at each time point of measurement (baseline, days 1 – 8), as well as for cumulative data (summation apatite release at baseline – day 8).

As the data was not normally distributed according to the Kolmogorov-Smirnov and Shapiro-Wilk tests, the non-parametric Kruskal-Wallis and Mann-Whitney tests were used to disclose differences between apatite release in the different groups at each time point of measurement.

To evaluate if the number of erosive, abrasive and remineralisations cycles had exerted an influence on the sealer permeability, the amount of apatite dissolved at the first and final day of the study were compared by the Wilcoxon Signed Ranks Test for each treatment group separately.

The results of the statistical analysis with p-values < 0.05 were considered to be statistically significant.

Results:

The amount of apatite dissolved during the erosive challenges performed at baseline, at days 1 – 8 of the groups 1 – 4, as well as cumulative apatite release (summation of apatite release during the erosive attacks at baseline and at days 1 – 8 of the respective samples) are presented in table 1.

At baseline measurement, the significantly highest apatite release was observed for the unsealed control group 1. The amount of apatite lost in the groups sealed with Seal&Protect, K-0184 and OptiBond FL (groups 2, 3 and 4) were not statistically significant different from each other at the baseline measurement.

At each day of the erosive, abrasive and remineralisation cycling (day 1 – day 8), the highest apatite loss was observed for the unsealed control group 1 ($p = 0.001$, respectively). The lowest apatite loss was observed for the samples sealed with Seal&Protect (group 2) and K-0184 (group 3), with no significant difference between these two groups. The samples sealed with OptiBond FL revealed a significantly lower apatite loss compared with unsealed samples (group 1), but a significantly higher loss when compared with samples sealed with Seal&Protect or K-0184 ($p = 0.001$, respectively).

The significantly highest cumulative apatite loss was observed for the unsealed samples of group 1 ($102.82 \pm 17.98 \mu\text{g}$). For the samples sealed with OptiBond FL (group 4), a significantly lower cumulative apatite loss was observed compared with the apatite loss of the unsealed samples (group 1). Compared with the apatite loss of the samples sealed with Seal&Protect or K-0184, a significantly higher cumulative apatite loss was observed for the samples sealed with OptiBond FL. The difference in cumulative apatite loss between groups 2 (Seal&Protect) and 3 (K-0184) was not statistically significant.

Within group 1 (unsealed control) and group 4 (OptiBond FL), the apatite loss at day 8 was not significantly different compared with the apatite loss at day 1. However, in groups 2 (Seal&Protect) and 3 (K-0184), the apatite loss at day 8 was significantly lower compared with the apatite loss at the day 1 ($p = 0.002$ and 0.005 , respectively).

Discussion:

In the present study, bovine dentine was used as a substitute for human dentine. Although it might be more clinically relevant to use human dentine, the use of bovine dentine has been well established in numerous studies investigating dentine wear by either tooth brushing [27,28], erosion [29,30] or combination of both [31,32]. Furthermore, it seems to be acceptable to use bovine dentine especially when the values of the respective test groups are compared with each other and with the untreated controls of the same study (relative values) [33].

Hydrochloric acid was used to simulate the erosive attacks caused by gastric juice. It is well known that beside hydrochloric acid [34], the gastric juice also contains various enzymes, including the proteolytic enzyme pepsin [35]. The results of studies [36,37] using pepsin admixture to hydrochloric acid for erosion simulation are inconclusive. Therefore, in the present study, the erosive attack was performed with pure hydrochloric acid, as performed in numerous other studies [12,14,19,24,38,39].

For the simulation of the toothbrush abrasion, fluoridated toothpaste as an abrasive was used. On one hand this might have an influence on the later wear, as it is well known that prior use of fluoridated toothpaste is able to reduce the erosive demineralisation of dentine/enamel [3]. However, the use of fluoridated toothpaste is widely recommended for patients, if not to prevent erosion then certainly to prevent formation of carious lesions. It was therefore decided to use a fluoridated toothpaste to simulate clinical conditions as closely as possible.

One day of the in-vitro cycling used in the present study simulates about one month (30 days) in-vivo, following the assumptions by Wiegand and Attin [33] that 10 - 15 brushing strokes per tooth during a single tooth brushing session being adequate to simulate the clinical condition in vitro. In 1996, Bartlett et al. [40] found a drop of the oral pH below 5.5 for 0.3% and below pH 6 for 4.4% of the total time during 24-hour pH telemetry in gastro-oesophageal reflux patients. This corresponds to an erosion time between 4.3 and 60 min per day, respectively. In light of these findings, an erosive time of 6 min with hydrochloric acid pH 3.0 seemed to be adequate to simulate one in-vivo day. As one day of the present erosive, abrasive and remineralisation cycling should simulate one month in-vivo, an erosive time of 3 h was chosen (6 min erosion/day in-vivo x 30 days = 180 min = 3 h).

Limitation of the present study might be that the remineralisation time (storage in artificial saliva) was proportionally low compared to the demineralisation time used. However, it might be assumed that no fundamental difference in the protective effect of the surface sealants against erosive demineralisation will be observed when longer remineralisation times would have been implemented. If a longer remineralisation time would have been used, it might be conceivable that a longer duration of the experimental procedure, which means a higher number of erosive/abrasive cycles, is needed until significant differences in the anti-erosive effect of the sealants may be observed. A further limitation might be that the samples in group 4 were etched with orthophosphoric acid before application of the bonding system. This etching leads to the loss of minerals from the dentine. During this loss 32P will also be lost, which cannot be detected in the acid after subsequent erosive attacks. This might result in an underestimation of apatite loss during erosion. However, we assume that this possible underestimation will not fundamentally change the findings of the present study, as the apatite loss in the group with the orthophosphoric acid etching (group 4) was already the highest of the sealed groups.

The hypothesis of the present study that an increasing number of erosion/abrasion cycles results in a reduced erosion-preventing efficacy of the tested materials was rejected. None of the tested surface sealants showed a significant increase in the apatite released during the erosive attacks of the erosive/abrasive cycling. This finding might be interpreted as a durability of the erosion-preventing efficacy of the tested sealants for observation period (simulating eight month in-vivo) used in this study. However, under clinical conditions, mechanical challenges other than erosion and toothbrush abrasion (demastication, attrition and abfraction) might also affect the surface sealants. Therefore, a shortened durability of the anti-erosive effect of the sealants in the clinical situation may be expected. This assumption is in accordance with the findings of Sundaram et al. (2007)[41] showing a protective effect of Seal&Protect against palatal tooth wear in patients suffering from erosion and/or bruxism for 6 – 9 month.

For the groups sealed with Seal&Protect and K-0184 a significantly lower apatite loss during the erosive attack on day 8 was observed when compared with the apatite loss during day 1 erosion. It would be a misinterpretation of the data, to interpret this finding as a result of an improvement of the erosion-preventing efficacy of theses sealants during the erosion/abrasion cycling. This reduction of the apatite release has to be attributed to certain material properties. Both of these sealants interact with the dentine surface during application like self-etch adhesives, diluting and incorporating minerals from the dentine surface in the sealants. Thus, in the present study, the sealants will also incorporate radioactive ^{32}P while reacting with the dentine, which can be diluted from the sealant's surface during the later performed erosive attacks. This ^{32}P will be primarily found in the acid of the first attacks resulting in a higher apatite wear at the first day. A possible incorporation in the oxygen inhibition zone of the sealants alone can be excluded, as the sealants were tooth brushed before the erosive attack of the first day.

The experimental sealant (K-0184) used in this study has the same chemical composition as the commercially available Seal&Protect, with the exception of triclosan. Triclosan is incorporated as an antimicrobial additive in numerous personal care and sanitizing products such as soaps, household cleaners, cosmetics, sportswear, mouthwash and toothpaste [42]. Recent studies have shown that triclosan is able to induce antibiotic resistances in various bacteria strains [43], to accumulate in human milk samples and in fish exposed to municipal wastewater [44], to be found in biosolids which might be spread on agricultural land (also causing adverse effects in the soil environment [45]) and to reduce serum thyroid hormone levels following oral administration [46]. Due to these findings, concerns about the use of triclosan have been raised. To avoid the possible negative side effects of triclosan, it would be judicious to forgo the addition of triclosan in products to be used in human subjects. As no significant differences in either the apatite released during the erosive attack of the baseline measurement and during the consecutive erosive attacks of days 1 - 8 or in the cumulative apatite losses for samples treated with Seal&Protect and K-0184 were observed, one might conclude that the absence or presence of triclosan in the sealants used had no significant effect on either its mechanical stability during erosion/abrasion cycles or its erosion-preventing efficacy. Furthermore, the above-mentioned finding that the apatite loss is reduced from day 1 to the day 8 in both groups, sealed with Seal&Protect and K-0184, supports this assumption.

The permeability (apatite release) of OptiBond FL was at all days during the erosion/abrasion cycles and also cumulatively higher than that of Seal&Protect. The reasons for this have to be attributed to differences in the composition of the two materials. In OptiBond FL, the size of the incorporated filler is much larger (0.6 μm) as compared with Seal&Protect (7 nm). It might be imaginable that during brushing superficial fillers may break out of the sealant. If one of the larger fillers in OptiBond FL were lost from the sealant, the resulting defect might result in an insufficiently sealed surface area, while for Seal&Protect an

equivalent loss of smaller fillers would presumably not result in an insufficient seal. However, as the apatite loss was constant for the duration of the erosive/abrasive cycling for both Seal&Protect and OptiBond FL, it might be assumed that the loss of fillers is limited to the beginning of the cycling. Another possible reason for the lesser erosion-preventing efficacy of OptiBond FL might be found in the filler content of both materials. The filler content of OptiBond FL (47 % filler load, manufacturer's information) is higher than that of Seal&Protect (10 wt% filler load, manufacturer's information), resulting in a higher viscosity, which might result in a lower wettability. This lower wettability might cause a less perfect seal of the dentine surface, leaving certain areas insufficiently sealed.

Conclusion:

The findings of the present study show that the surface sealants applied were able to reduce the erosive apatite loss and maintain an erosion-preventing efficacy over the entire duration (simulating 8 month in-vivo) of the erosive/abrasive cycling.

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Table legends:

Tab. 1: Mean values (95% confidence interval) of apatite release (in μg) for each group and time point of measurement (baseline, days 1 -8) and cumulative apatite release (summation baseline – day 8)(S&P = Seal&Protect, K-0184 = experimental sealer and OB = OptiBond FL).

Within the same time point of measurement, values for different groups, which are not statistically significantly different, are marked with identical superscript capitals (read vertically).

Within the same groups, comparisons of apatite release at 1. and 8. day that are statistically significantly different, are marked with asterisk (read horizontally).

Group	Sealer	Time point of measurement									Cumulative
		Baseline	1. day	2. day	3. day	4. day	5. day	6.day	7. day	8. day	
1	-	26.99 A (22.23/31.74)	11.06 A (8.51/13.61)	8.91 A (5.85/11.97)	8.17 A (5.30/11.04)	8.51 A (5.81/11.23)	7.99 A (5.31/10.66)	10.36 A (7.35/13.37)	10.62 A (6.73/14.51)	10.21 A (6.49/13.93)	102.82 A (91.40/114.25)
2	S&P	0.75 B (0.51/0.98)	0.11 C * (0.07/0.16)	0.09 C (0.02/0.16)	0.07 C (0.00/0.15)	0.03 C (0.01/0.04)	0.03 C (0.01/0.04)	0.03 C (0.01/0.05)	0.05 C (0.02/0.12)	0.02 C * (0.00/0.05)	1.18 C (0.73/1.62)
3	K-0184	0.72 B (0.52/0.93)	0.08 C * (0.05/0.10)	0.08 C (0.04/0.13)	0.04 C (0.02/0.06)	0.02 C (0.01/0.03)	0.02 C (0.01/0.02)	0.04 C (0.01/0.07)	0.02 C (0.01/0.03)	0.02 C * (0.01/0.03)	1.04 C (0.81/1.27)
4	OB	1.02 B (0.76/1.28)	0.37 B (0.13/0.60)	0.17 B (0.08/0.26)	0.14 B (0.06/0.22)	0.14 B (0.06/0.22)	0.12 B (0.06/0.19)	0.14 B (0.07/0.21)	0.14 B (0.06/0.21)	0.23 B (0.14/0.33)	2.47 B (1.66/3.28)

Tab. 1: Mean values (95% confidence interval) of apatite release (in µg) for each group and time point of measurement (baseline, days 1 -8) and cumulative apatite release (summation baseline – day 8)(S&P = Seal&Protect, K-0184 = experimental sealer and OB = OptiBond FL).

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Group	Sealer	Time point of measurement									Cumulative
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2	S&P	0.75 B (0.51/0.98)	0.11 C * (0.07/0.16)	0.09 C (0.02/0.16)	0.07 C (0.00/0.15)	0.03 C (0.01/0.04)	0.03 C (0.01/0.04)	0.03 C (0.01/0.05)	0.05 C (0.02/0.12)	0.02 C * (0.00/0.05)	1.18 C (0.73/1.62)
3	K-0184	0.72 B (0.52/0.93)	0.08 C * (0.05/0.10)	0.08 C (0.04/0.13)	0.04 C (0.02/0.06)	0.02 C (0.01/0.03)	0.02 C (0.01/0.02)	0.04 C (0.01/0.07)	0.02 C (0.01/0.03)	0.02 C * (0.01/0.03)	1.04 C (0.81/1.27)
4	OB	1.02 B (0.76/1.28)	0.37 B (0.13/0.60)	0.17 B (0.08/0.26)	0.14 B (0.06/0.22)	0.14 B (0.06/0.22)	0.12 B (0.06/0.19)	0.14 B (0.07/0.21)	0.14 B (0.06/0.21)	0.23 B (0.14/0.33)	2.47 B (1.66/3.28)

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